

Article

Effects of Swine Biogas Slurry on Arbuscular Mycorrhizal Fungal Community in Poplar Plantations on Northeast China Sandy Soils

Shuhui Li ^{1,2,†}, Weixi Zhang ^{2,3,†}, Hengming Zhang ⁴, Siqi Wu ^{1,5}, Keye Zhu ⁶, Changjun Ding ^{2,3,*} and Wenxu Zhu ^{1,5,*} 

¹ College of Forestry, Shenyang Agricultural University, Shenyang 110065, China; lishuhui@stu.syau.edu.cn (S.L.); wsq2020@syau.edu.cn (S.W.)

² State Key Laboratory of Tree Genetics and Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China; weixizhang@caf.ac.cn

³ Key Laboratory of Tree Breeding and Cultivation, State Forestry Administration, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China

⁴ Liaoning Academy for Environmental Planning Co., Ltd., Shenyang 110031, China; 15140059629@163.com

⁵ Research Station of Liaohe-River Plain Farmland Shelterbelt Ecosystem CEN, College of Forestry, Shenyang Agricultural University, Shenyang 110866, China

⁶ Co-Innovation Center for Sustainable Forestry in Southern China, Department of Forest Protection, College of Forestry and Grassland, Nanjing Forestry University, Nanjing 210073, China; ansizky@njfu.edu.cn

* Correspondence: changjunding@caf.ac.cn (C.D.); zhuwx@syau.edu.cn (W.Z.)

† These authors contributed equally to this work.

Highlights

What are the main findings?

- Biogas slurry and soil depth jointly shape AMF communities, elevating *Glomus* abundance and subsurface richness in sandy poplar soils.
- Soil phosphorus, nitrogen and pH are the dominant drivers of biogas slurry-induced AMF community shifts.

What are the implications of the main findings?

- These findings support site-specific fertilization and sustainable management of sandy poplar plantations.
- They provide a scientific basis for biogas slurry recycling in sandy forest ecological restoration.

Abstract

Arbuscular mycorrhizal fungi (AMF) are the key symbiotic microorganisms in sandy poplar plantations, regulating vegetation restoration and nutrient cycling. This study aims to investigate the impact of biogas slurry (BS) application on the community structure of AMF in the rhizosphere soil of poplar forests, in order to support the sustainable management of the forest. This study focused on poplar plantations in the sandy areas of Northeast China. High-throughput sequencing technology was utilized to examine the community structure and diversity characteristics of AMF while measuring the physical and chemical characteristics of the soil. The application of BS increased the nutritional status of poplar rhizosphere soil and changed its physical and chemical characteristics, according to the findings. *Paraglomus* and *Glomus* dominated the AMF community. The application of BS increased the relative abundance of *Glomus* in the 0–20 cm and 20–40 cm soil strata. The primary determinants of changes in the dominant AMF populations of poplar were TP, AP, TN, NO₃[−]-N, and pH. To conclude, BS application together with soil depth regulates AMF



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community composition within sandy poplar plantations. BS amendment ameliorates soil nutrient conditions, increasing subsurface AMF diversity and the relative abundance of vital functional genera. Three core hypotheses were tested: (1) BS application elevates soil carbon, nitrogen and phosphorus concentrations. (2) Biogas slurry and soil depth jointly alter AMF richness and composition. (3) BS-induced shifts in soil nutrients are significantly correlated with AMF community assemblage.

Keywords: arbuscular mycorrhizal fungi; high-throughput sequencing; afforestation; microorganisms; plant–microbe interactions

1. Introduction

Roughly 35% of the world's geographical surface is made up of arid and semi-arid areas [1,2]. China is one of the nations most at risk from desertification in the world, with 52.5% of its land covered by dry and semi-arid areas [3]. The sandy areas in Northeast China, the core region of northern China's desertification control, have long suffered from wind erosion and desertification, soil nutrient loss caused by water and soil erosion, and declining land productivity. These issues have severely restricted regional ecological security and social economic development [2]. Afforestation is one of the key means of desertification control. Among them, poplar trees, due to their strong adaptability and fast growth rate, have become one of the main tree species for artificial afforestation in the sandy areas of Northeast China. They play an irreplaceable role in wind prevention and sand fixation, water and soil conservation, and carbon sequestration [4,5].

Since the 1950s, China has carried out large-scale tree-planting in sandy zones including western Northeast China and eastern Inner Mongolia via bioengineering for ecological rehabilitation [6]. Subsequently, a series of forestry ecological projects including the Three North Shelter Forest Project were implemented, and the area of artificial forests represented by poplar trees continued to expand, significantly enhancing the total forest resources and ecosystem service functions in the region [7,8]. However, with the long-term operation of poplar artificial forests, a series of problems have gradually emerged: some forest stands have experienced decline due to insufficient consideration of water resource-carrying capacity during the initial construction, excessive planting density, and single tree species, resulting in frequent diseases and pests, and a decline in windbreak and sand fixation functions, which has restricted the continuous realization of the ecological benefits of artificial forests [9,10].

Soil properties are thought to be the primary drivers of plant growth and preservation of ecosystem services during the vegetation restoration process of artificial forests in sandy environments [11,12]. The soil microbial community can reflect the dynamic changes in soil quality more accurately than the physical and chemical characteristics of the soil. As the central group of plant–microbe interactions, mycorrhizal fungi are essential for boosting plant resistance, facilitating nutrient absorption, and preserving ecosystem stability [13–15]. Microorganisms occupy different ecological niches and exert different ecological functions. Therefore, microbial diversity is the foundation for maintaining soil ecological functions [16–18]. Prior research has demonstrated that the variety of the soil microbial population is significantly impacted by the application of BS [19]. Xu et al. [20] used soil from the rice–rape rotation system as the research material and explored the effect of pure BS application on soil microbial diversity. The results showed that the application of BS increased the richness of soil fungal community.

AMF are a key fungal group in the fungal kingdom that can form symbiotic associations with plants. Over 90% of vascular plants can form mycorrhizal symbioses with them [21]. During the formation of arbuscular mycorrhiza, when the activity of the plant root meristem is inhibited, the symbiont will adopt a series of adaptive strategies to reshape the root morphology. For example, it promotes the increase in the number of adventitious roots and lateral roots, thereby expanding the absorption surface area of the roots and enhancing the vitality of the roots, ultimately achieving the purpose of promoting the absorption and utilization of water and nutrient elements by the plant [22]. Moreover, arbuscular mycorrhiza has a close connection with the host plant in the utilization of various nutrients, including phosphorus, nitrogen, carbon, trace mineral elements, and plant endogenous hormones. They can activate the mineral nutrients in the soil and promote the absorption of mineral elements by the plant. Simultaneously, the mycorrhizal fungi can improve the rate at which soil organic matter (OM) decomposes and their capacity to absorb nitrogen, which would further activate the phosphorus elements in the soil and create a more favorable nutritional environment for plant growth [23,24]. In addition, studies have shown that AMF are also crucial in protecting plants from pathogens, drought, heavy metals, and salt stress. Their secretions are crucial to the growth and development of the host plant because they can change insoluble materials in the soil, encourage vegetation recovery, and enhance ecosystem stability [25–27].

BS as an organic soil amendment, contains nutrients such as nitrogen, phosphorus, and potassium [28,29]. It is also rich in amino acids, growth hormones, and effective microorganisms. Plant growth and stress tolerance can both be improved by this nutrient-rich slurry [30,31]. Additionally, it may enhance the soil's biological, chemical, and physical characteristics. For example, it can enhance soil aeration, water retention, stability, and by regulating the soil pH, improve soil aggregation and increase total nitrogen and OM content [32,33]. Additionally, BS contains a large amount of soluble inorganic phosphorus, which is a valuable phosphate fertilizer [34]. The majority of research has demonstrated that applying BS directly improves soil fertility [35], which can increase soil nutrient content, improve soil physical and chemical properties [36], and effectively regulate ratios of various nutrients in the soil [37]. Therefore, in some countries, BS is widely used as a fertilizer substitute in agricultural soil to improve soil fertility or increase crop yields [38,39].

According to reports, the AMF community may be impacted in certain agricultural areas by the increase in effective nutrients brought about by the addition of exogenous nutrients. This is because, in the absence of soil nutrient restrictions, plants will naturally direct resources toward other restricted components, which will lead to a decrease in fine roots and mycorrhizal structures [40]. Additionally, research has demonstrated that using nitrogen fertilizers can change the species richness and diversity of AMF and decrease AMF biomass [41,42]. However, the impact of BS application on the characteristics of AMF community in the artificial poplar forest of the Northeast sandy land remains unclear. Therefore, it is crucial to comprehend how AMF's structure and composition change following BS application in order to use AMF to increase soil nutrient efficiency. We put out the following theories: (1) Applying BS can enhance soil nutrients. (2) Applying soil biogas slurry and soil depth together change the taxonomic composition and richness of AMF. (3) There is a strong correlation between the compositional structure of soil AMF populations and the alterations in soil nutrients brought about by BS application. This study examined the composition and alterations of soil mycorrhizal fungi following BS application, which not only resolves the conflict between the treatment and use of BS and the scarcity of water and fertilizer resources in forestry and agriculture, but also accomplishes the goal of improving the soil quality of forest land in arid regions. Simultaneously, the findings of our study offer a scientific foundation for the use and safe treatment of BS.

2. Materials and Methods

2.1. Site Description

The study site is located at the Fu Jia Forest Farm in Changtu County, Tieling City, Liaoning Province, China ($42^{\circ}53'23''$ N– $43^{\circ}23'23''$ N, $123^{\circ}53'12''$ E– $123^{\circ}53'56''$ E), situated at the southeastern edge of the Horqin Sandy Land (Figure 1). The region belongs to the temperate subhumid continental monsoon climate zone. The frost-free season lasts 151 days; the mean annual precipitation is 656.5 mm; the annual active cumulative temperature over 10°C is 3348.3°C . Aeolian sandy soil is the most common form of soil. Deng et al.'s study included detailed information about the study area and sample plot settings [43].



Figure 1. Location of the study area.

2.2. Experimental Design and Soil Sample Collection

A randomized block design was employed within the plantations of *Populus pseudo-simonii* Kitag. at the Fujia Mechanized Forest Farm, with eight experimental plots established. Each plot covered an area of 800 m^2 ($20\text{ m} \times 40\text{ m}$), with horizontal and vertical intervals of 100 m between plots to minimize interference between adjacent treatments. Poplars were planted at a spacing of $2\text{ m} \times 6\text{ m}$, and the stand age was 21 years at the time of sampling. At a dosage of 525 m^3 per hectare per year for forest land, BS was applied to four experimental areas. The dosage was based on the research by Lai et al. [44], while the remaining four experimental areas used the same amount of water as a control treatment. The experiment was set up with four treatment groups, each with four replicates. Eight soil cores were randomly selected from the plot and evenly mixed to create each replicate. The BS samples were sourced from the nearby Mu Yuan Industrial Group Co., Ltd. (Nanyang, China). Basic physicochemical indicators of biogas slurry are presented in Table S1.

Details of the poplar experimental plots with biogas slurry application are shown in Figures S1 and S2. Three months following the application of BS, soil samples were taken in mid-July 2023. For each plot, soil cores were extracted from two soil levels (0–20 cm and 20–40 cm) using a soil auger with a diameter of 3 cm. Within each plot, eight random soil cores were mixed to form one composite sample per soil layer. This two-factor experiment contained two BS treatments (BS application/water control) and two soil depths, forming four combined experimental groups: PF1 (0–20 cm, BS), PF2

(20–40 cm, BS), PC1 (0–20 cm, control), PC2 (20–40 cm, control). Each group included four plot replicates, generating 16 composite soil samples in total. The samples were placed in sealed bags and brought back to the laboratory. After removing plant and animal residues and gravel, the soil samples were divided into two parts. pH, total carbon (TC), total nitrogen (TN), total phosphorus (TP), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), and accessible phosphorus (AP) were all analyzed in one part of the soil. The other portion was stored at $-80\text{ }^\circ\text{C}$ for DNA sequencing analysis.

2.3. Determination of Soil Properties

A benchtop pH meter (pH 700, Eutech Instruments Pte Ltd., Singapore) was used to measure the soil pH using the potentiometric method with a water-to-soil ratio of 2.5:1 [45]. An elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany) was used to measure soil TC and TN [46,47]. An AA3 flow analyzer (AA3, SEAL Analytical GmbH, Hamburg, Germany) was used to measure soil $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$. The molybdenum-antimony colorimetric method was used to measure TP in the soil. The sodium bicarbonate extraction molybdenum-antimony colorimetric method was used to measure soil AP [48].

2.4. DNA Extraction and PCR Amplification Sequencing

The MoBioPowerSoil[®] DNA Isolation Kit (MP Biomedicals, Santa Ana, CA, USA) was used to extract DNA from a sample that contained 0.5 g of fresh soil in accordance with the kit's instructions. To assess the extracted DNA's integrity, 1% agarose gel electrophoresis was used, and the NanoDrop ND-1000 nucleic acid (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the DNA's concentration and purity. Primers AMV4.5NF (AAGCTCGTAGTT-GAATTTCG) and AMDGR (CCCAACTATCCCTATTAATCAT) were used for the amplification.

An initial denaturation at $98\text{ }^\circ\text{C}$ for two minutes was followed by twenty-five cycles of denaturation at $98\text{ }^\circ\text{C}$ for fifteen seconds, annealing at $55\text{ }^\circ\text{C}$ for thirty seconds, extension at $72\text{ }^\circ\text{C}$ for thirty seconds, and a final extension at $72\text{ }^\circ\text{C}$ for five minutes. The Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) was used to quantify the PCR products after they had been purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China). The amplification products were then sequenced using the Illumina NovaSeq platform and NovaSeq 6000 SP Reagent Kit (500 cycles) from Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for $2 \times 250\text{ bp}$ paired-end sequencing.

2.5. Statistical Analysis

Raw data from high-throughput sequencing were initially processed using QIIME 2 (2019.4) [49]. The DADA2 plugin was then used to quality-filter, denoise, combine, and detect chimeras in the sequences [50]. Using non-singleton amplicon sequence variations (ASVs), a phylogenetic tree was created with the aid of fasttree 2 and mafftA to produce an ASV abundance table [51,52]. In order to equalize the sequencing depth bias beforehand, singleton ASVs were eliminated and the feature table was rarefied to the least sequencing depth across all samples. The MaarjAM reference database was used for the taxonomic assignment of AMF ASVs. The feature table was generated prior to running the 'qiime taxa barplot' command in QIIME 2 (2019.4). The taxonomic composition of each sample was visualized at the phylum and genus levels using this command; taxonomic profiles were examined at the class and genus levels for poplar-associated AMF. The results are shown in the corresponding bar charts. Using R's ggplot2 package (v3.2.0) and QIIME2 (2019.4), microbial alpha diversity indices were computed. To comprehensively evaluate the alpha diversity of soil and AMF communities, we characterized them using the Chao1 index [53], Shannon index [54], Simpson index [55], and Pielou_e index [56]. The significance of

these differences was verified using the Kruskal–Wallis rank sum test and Dunn’s post hoc test with Bonferroni adjustment for pairwise multiple comparisons. To determine the shared and unique ASVs among the experimental samples, Venn diagrams were created from the ASV abundance table using the VennDiagram package (v1.7.3) in R software (v4.2.1). Levene’s test was used to verify the homogeneity of variances before parametric analysis, and the Shapiro–Wilk test was employed to evaluate the normality of all datasets. Furthermore, substantial variations in the chemical characteristics of the soil following the application of BS were found using one-way analysis of variance (ANOVA). The notable variations in soil nutrient data produced in Microsoft Excel 2019 were analyzed using SPSS 26.0. The microbial community structure differences between samples were evaluated using the Bray–Curtis distance analysis technique (Bray and Curtis 1957) [57], and principal coordinate analysis (PCoA) [58] and non-metric multidimensional scaling (NMDS) were used for visualization.

3. Results

3.1. Effect of BS Application on Chemical Properties of Poplar Soils

The data in Table 1 illustrated that for the 0–20 cm soil layer, compared with the PC1 treatment, the application of BS significantly increased the contents of AP ($p < 0.01$), TN ($p < 0.001$), NO_3^- -N ($p < 0.05$), and TC ($p < 0.05$), as well as the pH value ($p < 0.001$), in the PF1. In particular, compared to the PC1 treatment, the PF1 soil’s TP, AP, TN, NO_3^- -N, and TC concentrations increased by 90.90%, 300.00%, 40.18%, 3527.23%, and 28.34%, respectively. Comparing the PC2 treatment with the 20–40 cm soil layer, the application of BS significantly affected AP ($p < 0.05$), TN ($p < 0.05$), NO_3^- -N ($p < 0.01$), and pH ($p < 0.001$), and these parameters increased by 400.00%, 46.43%, 2240.48%, and 8.90% respectively.

Table 1. Effects of applying BS on the chemical characteristics of various soil layers.

	Total P /g kg ⁻¹	Available P /mg kg ⁻¹	Total N /g kg ⁻¹	NH_4^+ -N /mg kg ⁻¹	NO_3^- -N /mg kg ⁻¹	Total C /g kg ⁻¹	C/N	pH
PC1	0.022 ± 0.006 b	0.60 ± 0.004 b	0.450 ± 0.002 b	126.34 ± 0.54 a	2.02 ± 0.53 b	4.983 ± 0.72 b	10.969 ± 0.334 a	5.02 ± 0.04 b
PF1	0.042 ± 0.009 a	2.40 ± 0.013 a	0.640 ± 0.003 a	135.43 ± 14.14 a	73.27 ± 25.34 a	6.395 ± 0.55 a	10.065 ± 0.347 a	5.59 ± 0.07 a
f	13.169	20.118	57.301	1.648	27.143	9.651	3.523	202.997
p	0.010	0.004	<0.001	0.247	0.002	0.021	0.110	<0.001
PC2	0.011 ± 0.007 a	0.12 ± 0.0001 b	0.280 ± 0.004 b	126.33 ± 2.22 a	2.94 ± 0.79 b	2.743 ± 0.16 a	10.053 ± 0.507 a	5.28 ± 0.05 b
PF2	0.021 ± 0.005 a	0.580 ± 0.0050 a	0.410 ± 0.002 a	141.20 ± 20.36 a	68.81 ± 25.16 a	3.563 ± 0.838 a	8.771 ± 0.583 a	5.75 ± 0.04 a
f	5.319	13.518	7.266	2.105	27.388	3.698	2.753	200.930
p	0.061	0.010	0.036	0.197	0.002	0.103	0.148	<0.001

The data in the table are average ± standard error (n = 4). Different small letters meant significant difference at 0.05 level. PC1: soil depth at 0–20 cm without BS application. PF1: soil depth at 0–20 cm with BS application. PC2: soil depth at 20–40 cm without BS application. PF2: soil depth at 20–40 cm with BS application.

3.2. Effect of BS Application on Composition of AMF in Poplar Stands

Figure 2 illustrates that a total of 3821 amplicon sequence variants (ASVs) were obtained. PC1, PC2, PF1, and PF2 owned 1562, 504, 1268, and 718 ASVs, respectively. The number of the shared ASVs among PC1, PC2, PF1, and PF2 was 77, and the unique ASVs were 1485, 427, 1191, and 641, respectively. PC1 and PC2 shared the most ASVs, whilst PC2 and PF1 shared the fewest.

As illustrated in Figure 3, Glomeromycetes and Paraglomeromycetes were found to be prevalent at the class level, with average relative abundances of 36.9% and 39.8%. Compared with PC1 and PC2, a tendency toward higher relative abundance of Glomeromycetes and lower relative abundance of Paraglomeromycetes was observed in PF1 and PF2 following BS application.

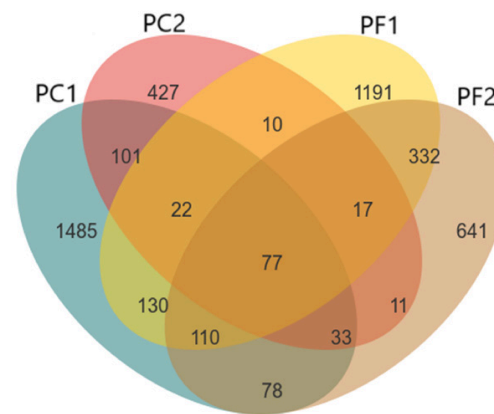
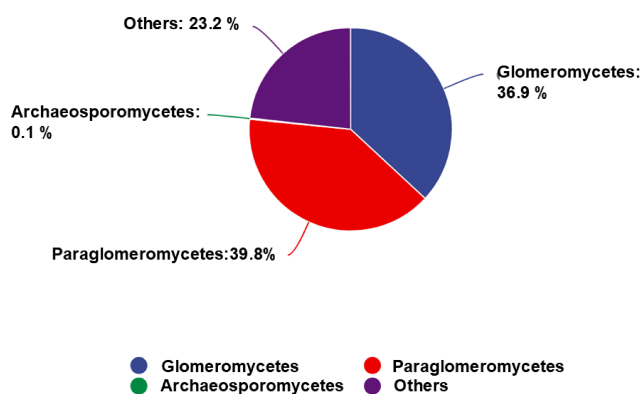
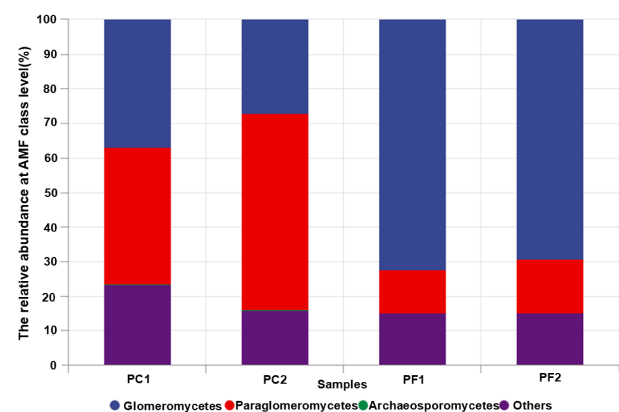


Figure 2. Venn diagram shows the unique and shared ASVs of four different poplar soil arbuscular mycorrhizal fungi. PC1: soil depth at 0–20 cm without BS application, PF1: soil depth at 0–20 cm with BS application, PC2: soil depth at 20–40 cm without BS application, PF2: soil depth at 20–40 cm with BS application.



(a)



(b)

Figure 3. Analysis of AMF community composition at the class level in *Populus*. (a) The average relative abundance of AMF; (b) the relative abundance of AMF.

With average relative abundances of 39.8% and 15.0%, respectively, *Paraglomus* and *Glomus* were prominent at the genus level, as shown in Figure 4. In the 0–20 cm layer of PF1 soil, trends of reduced relative abundance were noted for *Paraglomus*, *Archaeospora*, *Claroideoglomus*, *Ambispora* and *Scutellospora*, alongside an upward trend in *Glomus* relative abundance after BS application. For the 20–40 cm layer of PF2 soil, BS application coincided with apparent increases in *Glomus* relative abundance and declines in *Paraglomus*, *Archaeospora* and *Ambispora*.

3.3. Effects of BS Application on Diversity of AMF in Poplar Plantations

The diversity indices of AMF in the soil, including the Chao1 index, the Pielou_e index, the Shannon index, and the Simpson index, were analyzed. For the 0–20 cm soil layer, no significant differences were observed in Chao1, Pielou_e, Shannon and Simpson indices between the biogas slurry treatment (PF1) and the control (PC1). The Chao1 index of arbuscular mycorrhizal fungi associated with *Populus* was increased under BS treatment compared with PC1 and PC2. The data in Table 2 showed that, PC1 had the highest Shannon index (6.77) and Simpson index (0.96); PF1 had the highest Chao1 index (654.88) and the lowest Simpson index (0.88); PC2 had the highest Pielou_e index (0.734) and the lowest Chao1 index (209.4); PF2 exhibited the lowest Pielou_e index (0.616) and Shannon indices (5.44).

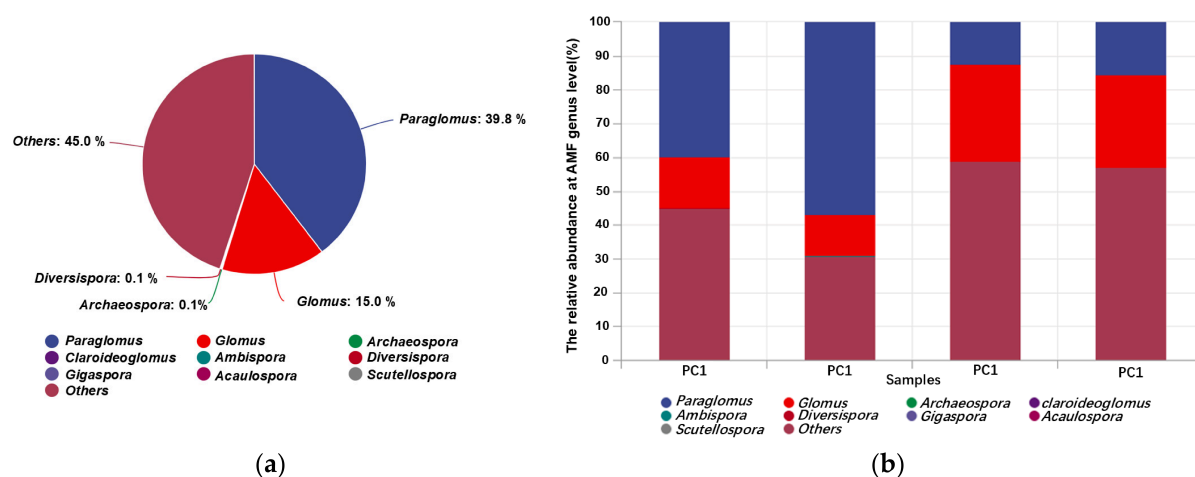


Figure 4. Analysis of AMF community composition at the genus level in *Populus*. (a) The average relative abundance of AMF; (b) the relative abundance of AMF.

Table 2. The difference analysis of diversity index of AMF.

	Chao1	Pielou_e	Shannon	Simpson
PC1	648.14 ± 65.30 A	0.728 ± 0.017 A	6.77 ± 0.14 A	0.96 ± 0.01 A
PF1	654.88 ± 99.56 A	0.632 ± 0.059 A	5.92 ± 0.69 A	0.88 ± 0.04 A
PC2	209.40 ± 30.58 B	0.734 ± 0.016 A	5.62 ± 0.22 A	0.92 ± 0.03 A
PF2	450.14 ± 61.20 A	0.616 ± 0.127 A	5.44 ± 0.60 A	0.89 ± 0.05 A

The data in the table are average ± standard error (n = 4). Different big letters meant significant difference at 0.01 level. PC1: soil depth at 0–20 cm without BS application. PF1: soil depth at 0–20 cm with BS application. PC2: soil depth at 20–40 cm without BS application. PF2: soil depth at 20–40 cm with BS application.

Figure 5 showed that the characteristics of poplar AMF communities differed between PC1 and PF1, and between PC2 and PF2. The PC1 and PC2 AMF communities were closely clustered in one category, and the PF1 and PF2 AMF communities were closely clustered in another category.

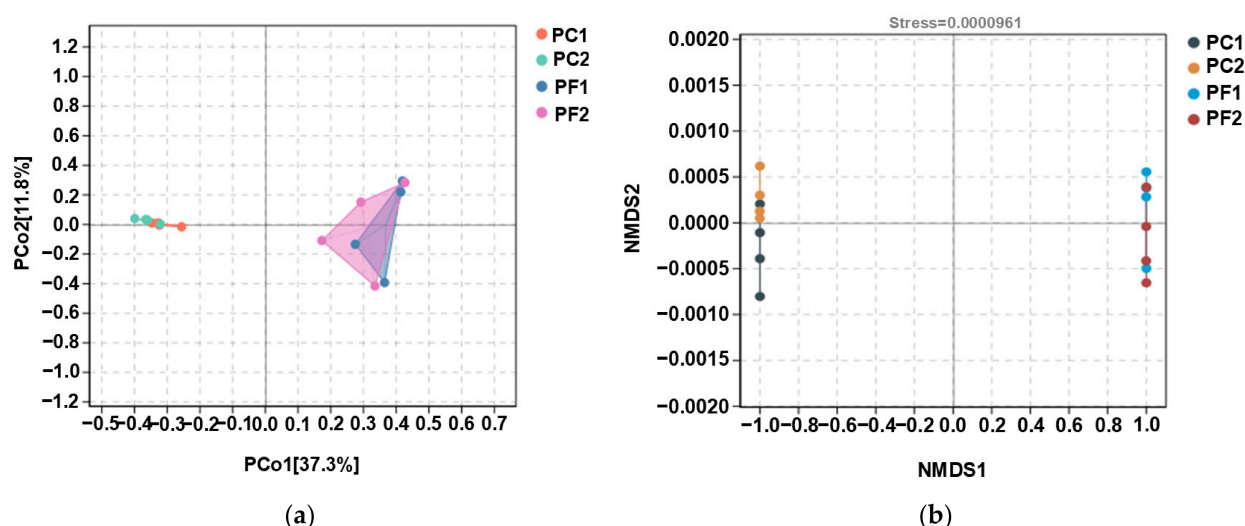


Figure 5. The analysis of differences in beta diversity of AMF. (a) Analysis of PCoA; (b) analysis of NMDS. PC1: soil depth at 0–20 cm without BS application, PF1: soil depth at 0–20 cm with BS application, PC2: soil depth at 20–40 cm without BS application, PF2: soil depth at 20–40 cm with BS application.

The application of BS had an effect on the AMF communities at various soil depths, according to the hierarchical cluster analysis at the genus level of AMF in the poplar forest

soil. In the control group, PC1 and PC2 were clustered into one branch, and in the test group, PF1 and PF2 were clustered into one branch, while PC1 and PF1 were obviously separated, and PC2 and PF2 were obviously separated. However, the samples from different soil depths before and after the application of BS did not separate (Figure 6).

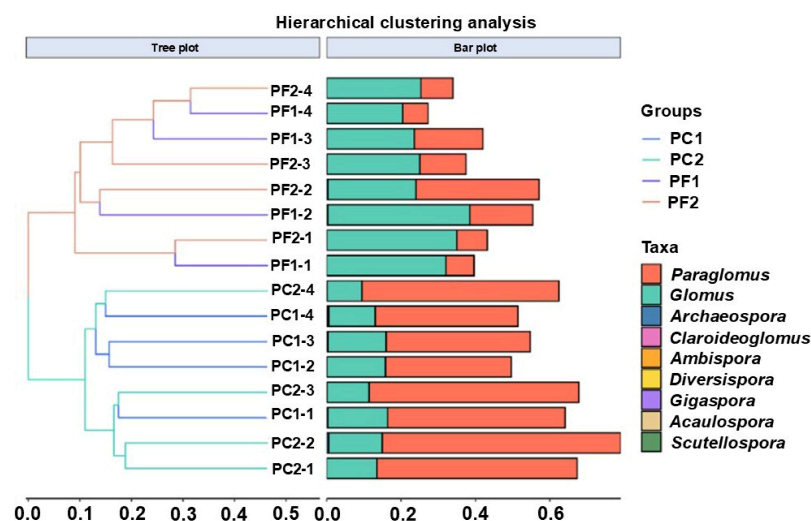


Figure 6. Horizontal hierarchical clustering analysis of AMF genera. The left side is a hierarchical clustering tree diagrams, where samples are clustered based on their similarity to each other. The shorter the branch length between samples, the more similar the two samples are. The right panel shows a stacked bar chart of the top 10 genera in terms of abundance.

3.4. Correlation of Community Composition and Diversity of AMF Soil Environmental Factors

Only *Paraglomus* and *Glomus* were significantly correlated with soil environmental factors. Among them, the genus *Paraglomus* was significantly correlated with TP ($p < 0.01$), AP ($p < 0.01$), TN ($p < 0.01$), $\text{NO}_3^- \text{-N}$ ($p < 0.01$), TC ($p < 0.05$), and pH ($p < 0.01$), whereas the genus *Glomus* was significantly correlated with TP ($p < 0.05$), AP ($p < 0.05$), TN ($p < 0.05$), $\text{NH}_4^+ \text{-N}$ ($p < 0.05$), $\text{NO}_3^- \text{-N}$ ($p < 0.01$), and pH ($p < 0.01$) (Figure 7).

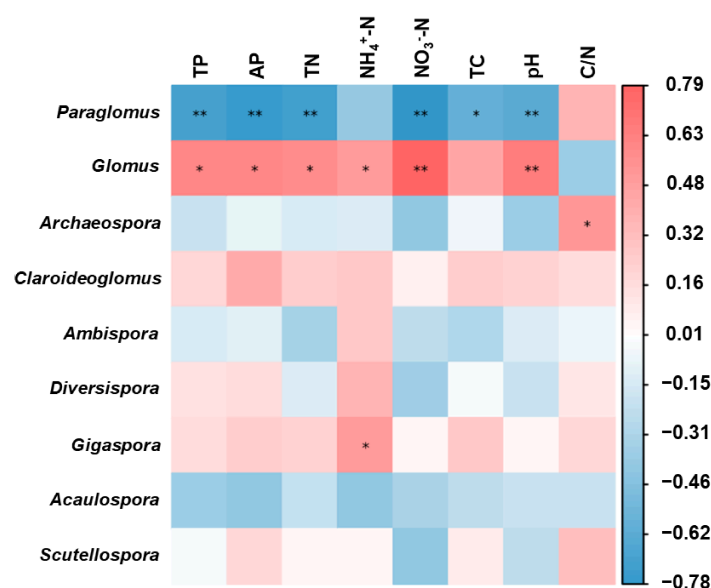


Figure 7. The Spearman correlation analysis between AMF community composition and soil environmental factors. * indicates a significant difference at the 0.05 level, while ** indicates a significant difference at the 0.01 level.

Chao1 index of poplar-associated AMF showed a highly significant positive correlation with soil TP ($r = 0.668$, $p = 0.005$), AP ($r = 0.744$, $p = 0.001$), TN ($r = 0.691$, $p = 0.003$), and TC ($r = 0.765$, $p = 0.001$) contents (Table 3). Soil Pieou-e index ($r = -0.524$, $p = 0.037$), Shannon index ($r = -0.550$, $p = 0.027$), and Simpson index ($r = -0.509$, $p = 0.044$) were significantly and negatively correlated with soil NO_3^- -N content. Notably, there was no significant correlation found between the diversity index of poplar arbuscular mycorrhizae and soil NH_4^+ -N, pH, and C/N.

Table 3. Spearman correlation analysis between soil environmental parameters and AMF diversity index.

	Chao1	Pielou_e	Shannon	Simpson
TP	0.668 **	−0.371	−0.041	−0.259
AP	0.744 **	−0.424	0.041	−0.165
TN	0.691 **	−0.432	0.009	−0.193
NH_4^+ -N	0.188	−0.065	−0.05	−0.100
NO_3^- -N	−0.050	−0.524 *	−0.550 *	−0.509 *
TC	0.765 **	−0.250	0.194	−0.059
pH	−0.109	−0.479	−0.459	−0.353
C/N	0.341	0.256	0.435	0.253

* denotes a significant association at the 0.05 level, while ** denotes a significant association at the 0.01 level.

4. Discussion

The results of this study indicated that the application of BS could improve the soil nutrient conditions of poplar trees, and the degree of impact on different nutrients varies. Moreover, the responses of different soil depths to the application of BS also differ. In previous studies, after applying BS, it supplements phosphorus to the surface soil, enhancing phosphorus availability [59]. The effects of phosphorus can extend to deeper layers, increasing soil phosphorus supply [60]. This is consistent with our research findings. Our research revealed that the application of BS increased the TP content in the 0–20 cm and 20–40 cm soil layers in comparison to the control group, and that the TP content in the 0–20 cm soil layer was higher than that in the 20–40 cm soil layer following the application of BS. The application of BS raised the rhizosphere soil's TN concentration in this study as compared to the control group. This is in line with other research findings that showed an increase in the rhizosphere soil's TN concentration following BS treatment [61]. In addition, compared with the control group, the application of BS significantly increased NO_3^- -N content in the rhizosphere soil of the 0–20 cm and 20–40 cm soil layers. This might be because NH_4^+ -N in the BS is easily nitrified and converted into more mobile soil nitrate nitrogen, thereby promoting nitrogen leaching [62]. The application of BS raised the overall carbon content in the 0–20 cm and 20–40 cm soil layers in this study when compared to the control group. Many studies have shown that the application of organic fertilizers will add additional carbon to the soil, increasing the soil carbon pool [63,64].

Our findings demonstrated that biogas slurry application modified the community structure of arbuscular mycorrhizal fungi in poplar rhizosphere. Fertilization is a widely adopted silvicultural strategy aimed at improving forest productivity. The diversity of AMF can affect the biodiversity and productivity of plant community [65]. Fertilization would decrease the AMF community's alpha diversity and lead the community to shift toward certain dominant species, according to Liu et al. [66]. However, Liu et al. [67] found that the alpha diversity of AMF was not significantly impacted by the use of organic fertilizer. This finding is in line with those of our present study. In this study, soil depth exerted a significant regulatory effect on AMF community richness ($f = 11.011$, $p < 0.01$). The impacts of biogas slurry on AMF richness varied with soil depth. Application of biogas slurry triggered a significant rise in the Chao1 index within the 20–40 cm subsoil, while the

0–20 cm topsoil showed an insignificant change. There were no remarkable differences in Pielou's evenness and Shannon diversity for both soil layers ($p > 0.05$). In this study, the AMF groups in the rhizosphere soil of poplar trees were mainly classified into three classes and ten genera. At the class level, all the fungal groups except for the unidentified ones and other groups accounted for more than 70% of the total, namely Glomeromycetes (36.9%), Paraglomeromycetes (39.8%), and Archaeosporomycetes (0.10%). At the genus level, these groups belonged to *Paraglomus*, *Glomus*, *Archaeospora*, *Claroideoglomus*, *Ambispora*, *Diversispora*, *Gigaspora*, *Acaulospora*, and *Scutellospora*. Among them, *Paraglomus* and *Glomus* were the dominant genera in the experimental area, which was in line with Yu et al.'s research findings [68]. Studies have shown that *Glomus* exhibits adaptability to ecological disturbances, has a wider distribution, and has a significantly higher number of spore individuals compared to other genera [69,70]. Therefore, it frequently contributes significantly to ecological processes including moderating interactions between AMF species [69,71].

Yu et al. [66] showed that adding BS to the rhizosphere soil of poplar trees can activate a wider range of AMF species. BS increases soil fertility and activates a greater variety of soil microorganisms than conventional inorganic fertilizers [72,73]. This is due to the fact that BS supplies more OM and must be broken down and changed by different microbes before it can be used, whereas conventional inorganic fertilizers, such as inorganic nitrogen and phosphorus fertilizers, can be directly absorbed by plants [74]. This is in accordance with our research results. Additionally, observational data indicated a tendency for higher relative abundance of the dominant genus *Glomus* following BS application in both the 0–20 cm and 20–40 cm soil layers. Research by Giovannetti et al. [75] and others has shown that *Glomus* mycelium exhibits distinct associative characteristics, with mycelium from this genus easily intertwining and aggregating. This unique biological trait confers the ability for *Glomus* AMF to reestablish new connection networks following human-induced mechanical disturbance. We also observed a tendency toward lower relative abundance of the mycorrhizal fungus *Paraglomus* following BS application. This indicated that the input of BS affected the composition of certain groups within the poplar AMF community structure. Additionally, different types of AMF exhibit varying degrees of tolerance and response to human disturbance [76], suggesting that there are differences in the life strategies of AMF.

This study found a substantial positive correlation between soil TC, TN, TP, and AP concentrations and the Chao1 index of AMF in poplar plants, similar to the results of Yu et al. [68]. Our research also showed that the evenness and general diversity of the AMF community in poplar trees were significantly negatively regulated by the amount of nitrate nitrogen in the soil. This result was in line with the meta-analysis of global nitrogen addition trials. This detrimental effect is directly linked to the buildup of nitrate nitrogen in the soil [77]. Under high soil nitrogen availability, host plants no longer rely on AMF to acquire nitrogen resources, and thus reduce the supply of photosynthetic carbon to AMF. This restrains the growth and reproduction of most AMF taxa. Nitrogen enrichment also reshapes the characteristics of AMF community, leading to simplified community structure and obvious decreases in species evenness and community diversity [78,79]. It is worth noting that soil $\text{NH}_4^+ - \text{N}$, pH, and C/N did not significantly correlate with the diversity index of poplar tree root-branch AMF in this investigation. This indicated that compared with the richness of the AMF fungal community, its diversity and evenness have a poorer correlation with soil environmental factors. The functional traits and ecological niche differentiation of different AMF species constitute the core mechanism by which they respond to differences in soil environments. Taxa with distant phylogenetic relationships often exhibit distinct environmental preferences [80]. The *Glomus* genus typically has a broad ecological niche and strong environmental adaptability, and can maintain a high abundance in high-nutrient soil environments [81], which was fully consistent with the

significant positive correlation between the *Glomus* genus and soil carbon, nitrogen, phosphorus nutrients, and pH in this study, while the *Paraglomus* genus is more inclined to colonize in low-nutrient and low-pH soil environments [81,82], which also explained the significant negative correlation between this genus and most nutrient factors and pH in this study. In summary, the taxonomic makeup of the AMF community in poplar rhizosphere soil is significantly linked with BS-induced changes in soil TC, TN, TP, AP, and NO_3^- -N.

There is evidence that the rhizosphere AMF community assembly and reproductive strategy were governed by soil OM acting as a dual filter. Exogenous OM mineralization, such as biogas slurry, continuously supplied labile C, N, and P substrates, expanding appropriate environments for generalist AMF taxa like *Glomus* and increasing AMF richness [68]. Nitrate buildup in soil may result from excessive nitrogen produced from over-decomposed organic materials [83]. The allocation of photosynthetic carbon from poplar hosts to their fungal symbionts was decreased by high N availability, which in turn inhibited the growth of some fungal taxa [84–86]. Notably, changes in soil nitrogen stoichiometry caused by OM further influenced the reproductive trade-off of AMF. OM addition increased available phosphorus and switched fungal growth toward reproductive development, promoting spore formation [87]; low-OM soils drove AMF to prioritize hyphal extension for nutrient acquisition with restricted propagule output [88,89]. Divergent feedbacks, however, were produced by genus-specific functional traits: under OM amendment, stress-tolerant *Glomus* formed interconnected hyphal networks that quickly regenerated propagules [90], whereas *Paraglomus* and *Ambispora* showed inhibited sporulation in high-OM, high-nitrate environments [91], which is consistent with our observed changes in genus relative abundance.

5. Conclusions

In summary, swine BS produces layer-specific effects on soil physicochemical properties and AMF in northeast sandy poplar plantations. BS significantly improved nutrients in both 0–20 cm topsoil and 20–40 cm subsoil with varying response magnitudes: total phosphorus and total carbon rose more in topsoil, while available phosphorus increased more in subsoil. Soil depth strongly regulated AMF richness—BS only significantly boosted Chao1 index in the 20–40 cm layer. Across both layers, BS raised the relative abundance of dominant genus *Glomus* and reduced *Paraglomus*. Beta diversity confirmed BS as the main driver of AMF community differentiation; total phosphorus, available phosphorus, total nitrogen, nitrate nitrogen and pH were key environmental filtering factors. This study clarifies the stratified coupling between rhizosphere AMF assembly and BS-altered soil nutrients, supporting stratified targeted fertilization and sustainable management of sandy poplar forests.

There are restrictions on this study. The results cannot be directly applied to other soil types, tree species, or climatic regions because they are based on a single site with sandy poplar plantations.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12080929/s1>, Figure S1: Field photograph of poplar plantation experimental plots for biogas slurry fertilization trial; Figure S2: Field photograph of biogas slurry application on poplar experimental plots; Table S1: Detection Table of Biogas slurry Composition.

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Abbreviations

The following abbreviations are used in this manuscript:

PC1	0–20 cm soil layer (no biogas slurry, equal-volume water control)
PC2	20–40 cm of soil layer (no biogas slurry, equal-volume water control)
PF1	0–20 cm soil layer (biogas slurry application treatment)
PF2	20–40 cm soil layer (biogas slurry application treatment)
AMF	Arbuscular mycorrhizal fungi
BS	Biogas slurry
NH ₄ ⁺ -N	Ammonium nitrogen
NO ₃ ⁻ -N	Nitrate nitrogen
TN	Total nitrogen
TC	Total carbon
TP	Total phosphorus
AP	Available phosphorus
OM	Organic matter

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